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Automated DNA, RNA, and Protein Extraction from Urine for Biobanking

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ABSTRACT

Introduction/Objective: DNA, RNA, and proteins are unavoidable human biomarkers. Today, blood remains the commonly used source of biomarkers despite numerous limitations. Therefore, other sources of biomarkers such as urine could be more appropriate for research in the field of bladder cancer. The aim of this study was to set up a new automated procedure for urinary DNA, RNA, and protein extraction and to evaluate their quality and quantity. **Materials and Methods:** This study was conducted in the setting of the COBLAnCE cohort. Urinary DNA and RNA were extracted using the Maxwell 16 system, and urinary proteins were isolated by precipitation from the supernatant and the cell pellet. The concentration and purity of nucleic acids were determined by spectrophotometry. RNA integrity was determined by the Agilent Bioanalyzer. PCR assays were also used to ensure the quality of DNA and RNA samples. The quality of protein samples obtained was determined by Western blot analysis. **Results:** PCR experiments performed highlighted that it is possible to use the DNA and RNA samples for amplification, gene expression, or genotyping. However, DNA and RNA recovery from urine was highly variable among patients, with a significant impact of the patient’s gender. The samples were highly degraded. Finally, our protocol of protein isolation was effective in extracting urinary supernatant proteins as well as pellet proteins. **Discussion:** Therefore, urine samples could constitute valuable resources for subsequent investigations in bladder cancer. These samples will allow identifying new easy-access biomarkers for the early detection of cancer, monitoring cancer progression, and assessing response to therapy.

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INTRODUCTION

Biomarkers are key players for providing valuable information for prevention, early detection, diagnosis, prognosis, and response to therapy of diseases. Their availability often requires easily accessible, good-quality human DNA, RNA, and proteins for molecular downstream applications.[1] Today, blood remains the main commonly used source of human biomarkers. However, it has several limitations, such as the need of a professional staff, equipment, and infrastructure for its withdrawal.[1] Therefore, the possibility of using other sources of biomarkers such as urine could be an interesting alternative.[2] Indeed, besides proteins filtrated and/or secreted by the kidneys, urine also contains nucleic acids[1],[3] and proteins[4] derived from the epithelial cells (renal tubular and urothelial cells), leukocytes, and also malignant cells, which are liberated spontaneously into urine. It is therefore mandatory to collect urine for prospective biological collections in the field of renal, bladder or prostate tumoral or nontumoral diseases, and extra-urological pathologies. It can be collected by a noninvasive method and does not require specialized staff, equipment, or infrastructure. It can be obtained in large volumes several times per day and therefore has advantages for large-scale studies.

Nowadays, large biological sample collections are often created within multicentric studies involving shipment and storage of various samples such as urine. The storage of the samples often occurs over a prolonged period and under various conditions before extraction of nucleic acids occurs, according to the storage possibilities of the participating centers (ambient temperature, +4°C, −20°C, or −80°C for days, weeks, or months). The time interval between sample collection and extraction of nucleic acid is often greater than 1 week. Hence, it is likely that these samples will be altered to some degree during storage before extraction of nucleic acids. Large-scale studies require a rapid, easy, and standardized protocol. Therefore, fully automated nucleic acid extraction approaches are highly convenient and recommended. Automated nucleic acid extractors have shown to be successful in extracting nucleic acids with efficient recovery, excellent reproducibility of the results, lack of cross contamination, and rapidity. Different human DNA extraction protocols have been reported in the literature for urine samples, eg, conventional manual methods, such as phenol-chloroform methods[1],[5],[6],[7],[8] that involve highly toxic reagents or standardized commercial DNA extraction kits.[9],[10],[11] Some data are also available concerning protein extraction procedures,[2],[12] and limited reports exist. Few data are reported in existing literature on urinary human RNA.

In this context, the goal of this study was to set up a new automated procedure for urinary DNA, RNA, and protein extraction and to ensure that their quality and quantity was suitable for molecular and biochemical downstream applications.

MATERIALS AND METHODS

Urine samples

This study was conducted in the setting of the COBLAnCE cohort, which has been reviewed and approved by an institutional ethics committee (CPP Ile de France VII, n°CO-12-001, 2012) and competent health authorities.[13] All patients gave written informed consent before participating to the study. Urine samples from healthy volunteers (13 women and 19 men for DNA extraction, 18 women and 14 men for RNA extraction, and 3 women and 6 men for protein extraction) and patients with bladder cancer (10 women and 47 men for DNA extraction, 11 women and 36 men for RNA extraction, and 4 women and 11 men for protein extraction) have been collected in 14 French hospitals participating in the study. It was required to collect mid-stream urine samples and to eliminate the first or last part. A total of 50 mL of these urine samples has been stored at room temperature in Norgen tubes (Norgen Biotek Corp) for nucleic acid extraction, and the urine surplus has been immediately aliquoted without centrifugation and frozen at −80°C in Falcon tubes for further protein extraction.

DNA and RNA extraction from urine samples

DNA and RNA were extracted using the Maxwell 16 system (Promega). A total of 25 mL of urine (Norgen tubes) was used for each DNA and RNA extraction. For DNA extraction, a Norgen tube was centrifuged 10 minutes at 2000*g* and the cell pellet was resuspended in 100 µL of PBS. A total of 300 µL of lysis buffer and 30 µL of proteinase K provided in the kit were added, and then the mixture was incubated 20 minutes at 56°C according to the manufacturer’s recommendations. After the lysis, the sample lysate was transferred to a Maxwell 16 LEV cartridge, and then the remaining purification process was fully automated by the extractor.

For RNA extraction, several strategies were tested in order to improve RNA quality: 1) washing the cell pellet to remove possible traces of urine and therefore the RNAses, 2) increasing the amount of thioglycerol used to neutralize RNAses contained in the urine sample, 3) adding an RNAse inhibitor during the RNA extraction, and 4) adding a protease inhibitor to the sample just after urine collection. Finally, based on the results of this pilot study, the validated method was the addition of 200 µL of a precooled (+4°C) 2% thioglycerol solution to the cell pellet obtained after centrifugation (10 min at 700*g*). A total of 200 µL of lysis buffer provided by the manufacturer was then added, and the lysate was transferred to a Maxwell 16 LEV cartridge. Purified DNA and RNA from the 25-mL urine samples were eluted in 50 µL nuclease-free water.

Purified DNA and RNA from the 25-mL urine samples were eluted from the paramagnetic beads in a final volume of 50 µL of nuclease-free water as per the manufacturer's protocol. Nucleic acid samples were then stored at −80°C until processed.

Protein extraction from urine samples

Urine samples stored at −80°C in Falcon tubes were thawed slowly at +4°C, homogenized, and centrifuged at 1300*g* for 10 minutes at room temperature. Then, proteins were isolated from both the supernatant and the obtained pellet separately. Proteins in the pellet were extracted by 200 µL of T-PER reagent (Tissue Protein Extraction Reagent, Thermo Fisher Scientific) containing protease and phosphatase inhibitors. After homogenization with beads, the sample was centrifuged at 10,000*g* for 10 minutes to eliminate the cell debris and stored at −80°C. In parallel, the supernatant was precipitated by trichloracetic acid 30% (Sigma-Aldrich) to reach a final concentration[2] of 6%, even if a precipitate was present in the supernatant after the thawing of the urine sample. The sample was mixed and incubated at 4°C. After a centrifugation at 14,000*g* for 15 minutes at +4°C, the supernatant was removed and the pellet was washed twice with ice-cold acetone (Sigma-Aldrich) to remove all interfering compounds. The supernatant was removed, and the pellet was air dried. Samples were stored at −80°C until use.

Concentration, purity, and quality assessment of DNA and RNA extracted from urine samples

Concentration and purity of nucleic acids (absorbance ratio at 260/280) were first determined by spectrophotometry using the NanoDrop 1000 (Thermo Fisher Scientific). An A260/A280 ratio below 1.7 is indicative of residual protein, phenol, or other reagents associated with the extraction protocol, whereas an A260/A280 ratio above 2.0 indicates RNA contamination. The concentration of DNA and RNA was also determined using Qubit fluorimeter assay (Thermo Fisher Scientific), according to the manufacturer's instructions. Indeed, it should be stressed that the NanoDrop quantification leads to an overestimation of the DNA concentration, whereas the Qubit quantification is specific to double-stranded DNA and thereby provides lower values than that measured with the NanoDrop.

RNA integrity was determined by the Agilent Bioanalyzer using the Agilent RNA 6000 pico kit (Agilent Technologies) according to the manufacturer's instructions in determining the RNA Integrity Number (RIN) and the DV300 (percentage of RNA fragments above 300 nucleotides). Real-time PCR (qPCR) and reverse-transcriptase (RT)-qPCR assays were also used to ensure the quality of DNA and RNA samples, respectively. For DNA, a first qPCR was performed on a housekeeping gene of TATA box binding protein (TBP) (119 bp) recommended as a reference for gene expression studies in human bladder cancer.[14] A total of 20 ng of extracted genomic DNA was used for qPCR using SYBR Green dye on the Applied Biosystems7000 real-time PCR system (Thermo Fisher Scientific). The following amplification conditions were used: 10 minutes at 94°C, followed by 40 cycles of 30 seconds at 94°C and 30 seconds at 60°C. The Cycle threshold (Ct) of TBP for each sample was determined. TBP primers 5’-CCAGACTGGCAGCAAGAAAAT-3’ and 5’-CCTTATAGGAAACTTCACATCACAGC-3’ were used. This allowed us to verify whether DNA extracted from urine samples was amplifiable. Thereafter, to complete the previous results, we performed a multiplex SNaPshot assay to detect the most frequent FGFR3 mutations in bladder cancer, localized in three exons (115 bp, 138 bp, and 160 pb).[15] RNA extracts were also tested for amplification by RT-qPCR using a reference gene, Cyclophilin A (99 bp), for which expression is commonly assumed to be invariable between cells of different samples and usually used as normalizer.[16] A total of 50 ng of RNA was used to generate cDNA by reverse transcription using the SuperScript VILO cDNA Synthesis kit (Thermo Fisher Scientific). qPCR was performed in the following amplification conditions: 10 minutes at 94°C, followed by 40 cycles of 30 seconds at 94°C and 30 seconds at 60°C using SYBR Green dye and the Applied Biosystems7000 Real-time PCR system (Thermo Fisher Scientific). The Ct of Cyclophilin-A for each sample was determined. Cyclophilin-A primers 5’-GTCAACCCCACCGTGTTCTT-3’ and 5’-CTGCTGTCTTTGGGACCTTGT-3’ were used.

Concentration and quality of proteins extracted from urine samples

The protein concentration was assessed with the BCA Protein Assay kit (Pierce), using Bovine serum albumine as a standard. The quality of protein samples obtained was determined by Western blot analysis. Two proteins were analyzed: uromodulin (UMOD polyclonal antibody, Abgent), which is a specific and abundant urinary protein, and TSG101 (monoclonal antibody, clone EPR7130(B), Abcam), which is an exosomal marker.

Statistical analysis

Statistical analysis was performed using a Student’s t test with significance at $P < 0.05$.

RESULTS

Both patients and healthy volunteers were included in the present study. For healthy volunteers, nucleic acids were extracted from fresh urine samples within 24 hours of collection. We also performed the extraction in different time points after collection in one healthy volunteer to evaluate the effect of storage time on RNA and DNA quality. Urine samples of COBLAnCE patients were stored in Norgen tubes at room temperature for an average time of 2.3 ± 1.9 months (range: 0.0 to 11.9 mo) before DNA and RNA extractions.

Purity of DNA extracted from urine samples

Based on the ratio of A260/A280, the purity of the DNA extracts was evaluated for both volunteers and patients, and the corresponding values are shown in Table 1. Of the 32 and 57 samples that were processed from healthy volunteers and patients, respectively, 44% and 36% exhibited a mean purity ratio (A260/A280) above 1.7. No statistically significant difference was detected in the A260/A280 ratio between patients with bladder cancer and healthy volunteers or between male and female individuals, suggesting that the purity of DNA samples was similar among groups. However, although no difference was highlighted in healthy volunteers, DNA concentration assessed by NanoDrop was higher for women's bladder cancer samples compared with men ($P < 0.05$).

Table 1

Purity of DNA samples determined by spectrophotometry

n		**Median ratio A260/A280**	**Median ratio A260/A230**	**Median concentration (ng/µL)**
Healthy volunteers	32	1.59 (1.19 -2.09)	1.05 (0.42-2.12)	28.9 (13.3-3946.8)
Male	19	1.46 (1.19-2.09)	0.91 (0.42-2.12)	21.9 (13.3-3946.8)
Female	13	1.74 (1.28-2.04)	1.32 (0.84-1.80)	173.2 (15.7-2906.7)
COBLAnCE patients	57	1.48 (0.44-2.42)	0.66 (0.40-0.96)	25.6 (6.6-513.3)
Male	47	1.51 (0.44-2.42)	0.63 (0.40-0.90)	23.3 (6.6-258.0)
Female	10	1.49 (0.55-1.94)	0.86 (0.53-0.96)	58.2 (16.2-513.3)*

*Values are median (range). *P < 0.05 versus male.*

Purity and integrity of RNA extracted from urine samples

Several strategies were tested on urine samples to improve RNA quality: 1) washing the cell pellet to remove possible traces of urine and, therefore, the RNAses, 2) increasing the amount of thioglycerol used to neutralize RNAses contained in urine samples, 3) adding an RNAse inhibitor during the RNA extraction, and 4) adding a protease inhibitor to the sample just after urine collection. Condition 1 provided the best extraction yield and RNA quality. This approach requires washing the cell pellet to remove possible traces of urine and, therefore, the RNAses (Table 2).

Table 2

Results of the series of experiments conducted to optimize the protocol for RNA extraction

	Median ratio A260/A280	**Median ratio A260/230**	**Median concentration (ng/µL)**	**Median RNA yield (µg)**	**RIN**
Condition 1 Washing cell pellet	1.58 (1.32-2.30)	0.98 (0.71-1.07)	7.4 (5.9-45.1)	0.4 (0.3-2.3)	4.2 (2.2-7.0)
Condition 2 Thioglycerol x 2	1.31 (0.51-1.66)	0.73 (0.41-0.90)	8.8 (4.7-13.8)	0.4 (0.2-0.7)	3.5 (2.8-8.5)
Condition 3 RNAse inhibitor	1.22 (1.10-1.59)	0.76 (0.52-0.94)	6.5 (5.8-15.9)	0.3 (0.3-0.8)	2,5 (1.5-5.6)
Condition 4 Protease inhibitor	1.39 (1.09-1.67)	0.96 (0.85-3.00)	5.1 (4.3-7.0)	0.3 (0.2-0.4)	2.5 (1.3-2.7)

n = 4 for each condition. Urine samples were collected in healthy female volunteers.

The A260/A280 ratios for the RNA extracts are shown in Table 3. Of the 32 and 47 samples that were processed from healthy volunteers and patients, respectively, 31% and 15% exhibited a ratio above 1.7. No statistical difference in this parameter was observed among groups, but, as observed for DNA samples, RNA concentration assessed by spectrophotometry was higher for female samples compared with male samples in patients with bladder cancer ($P < 0.05$). Finally, the RIN score assessed with Bioanalyzer highlighted that RNA extracted from urine samples was very degraded, as the RIN score was below 7 for all samples, except in 3 and 6 samples obtained from healthy volunteers and COBLAnCE patients, respectively. Figure 1 shows examples of electrophoretic profiles and DV300 of RNA extracted in COBLAnCE patients (Figure 1).

Table 3

Purity of RNA samples determined by spectrophotometry and integrity of RNA samples determined by RIN

	n	**Median ratio A260/A280**	**Median ratio A260/A230**	**Median concentration (ng/µL)**	**RIN**
Healthy volunteers	**32**	1.52 (1.09- 2.53)	0.85 (0.41-3.00)	7.0 (4.3-45.1)	2.6 (1.0-8.5)
Male	14	1.41 (1.09-1.92)	0.74 (0.41-0.98)	6.5 (4.3-13.8)	2.7 (1.0-8.5)
Female	18	1.63 (1.10-2.53)	0.94 (0.52-3.00)	7.7 (4.3-45.1)	2.6 (1.2-5.6)
COBLAnCE patients	**47**	1.41 (0.57-1.92)	0.63 (0.38-0.96)	10.0 (3.0-394.9)	2.4 (1.0-9.8)
Male	36	1.40 (1.19-1.84)	0.63 (0.38-0.91)	10.0 (3.0-50.0)	2.4 (1.0-8.8)
Female	11	1.53 (0.57-1.92)	0.63 (0.47-0.96)	15.6 (4.4-394.9)*	2.6 (1.0-9.8)

*Values are median (range). *P < 0.05 versus male.*

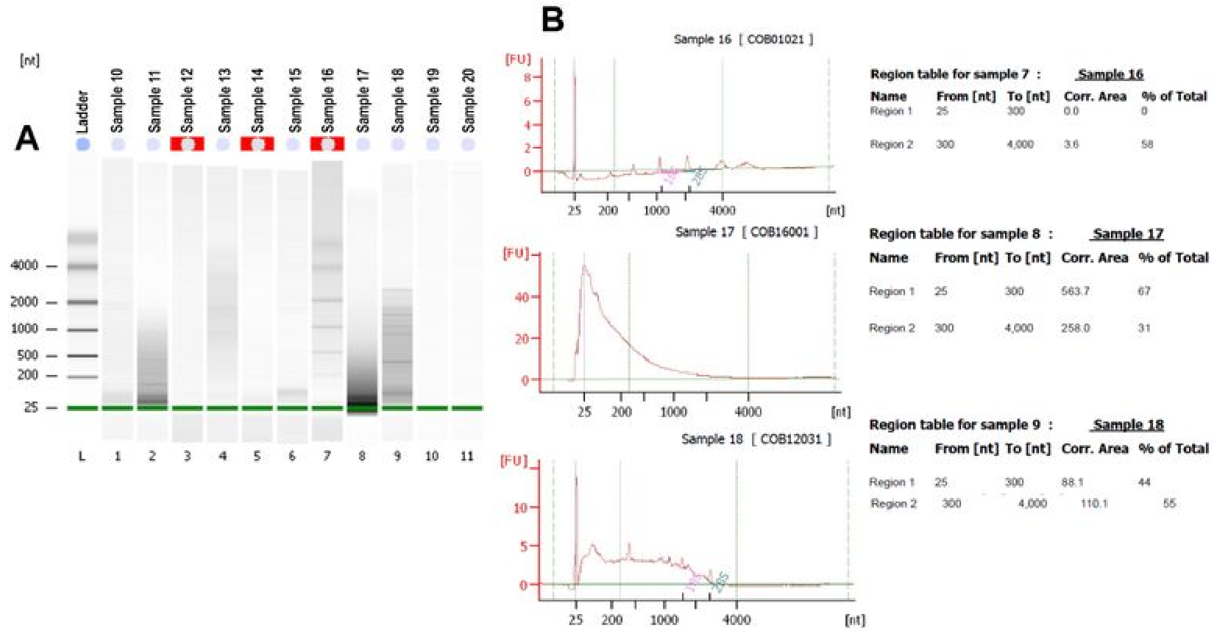

Figure 1

(*A*) Electrophoresis summary of 11 RNA extracted from urine of COBLAnCE patients. (*B*) Examples of DV300 for a slightly degraded RNA (sample 16: 58% of fragments of >300 nucleotides), moderately degraded (sample 18: 55% of fragments of >300 nucleotides), and much degraded (sample 17: 44% of fragments of >300 nucleotides).

Concentrations of DNA and RNA extracted from urine samples

We evaluated the concentration of total extracted DNA and RNA, as obtaining the highest DNA quantity is of major importance for biobanking. Table 4 summarizes the concentration of DNA samples assessed by fluorimetry. DNA concentrations were higher in female samples compared to male in COBLAnCE patients ($P < 0.05$). For RNA extracts, the fluorimetric concentrations could not be determined, as concentrations determined by spectrophotometry were lower than the limit of quantification (<2 ng/mL) required by the fluorimetric method.

Table 4

Concentration of DNA samples determined by fluorimetry

	n	**Median concentration (ng/µL)**
Healthy volunteers	**32**	20.9 (0.6-1100.0)
Male	19	5.4 (0.6-1100.0)

Female	13	39.2 (0.6-360.0)
COBLAnCE patients	**18**	4.8 (2.1-154.6)
Male	13	3.4 (2.1-74.2)
Female	5	84.6 (2.2-154.6)*
*Values are median (range). *P < 0.05 versus male.*		

Amplification and determination of the presence of PCR inhibitors

To verify the quality of DNA and RNA extracts, PCR experiments were conducted to determine whether the extracted nucleic acids were amplifiable and to ensure that no PCR inhibitors were coeluted during the extraction. Table 5 shows the results of the qPCR conducted using the reference gene in human bladder cancer TBP for DNA and the reference gene Cyclophilin A for RNA. The results of the multiplex SNaPshot assay performed on urine DNA of 40 COBLAnCE patients showed that 4 patients harbor mutations of *FGFR3*, 2 with S249C mutation and 2 with Y375C mutation, suggesting that the quality of DNA extracted is satisfactory to detect genetic alterations (Figure 2).

Table 5

Results of DNA and RNA qPCR

qPCR	Gene	Median C_t	n with $C_t > 30$	n with $C_t < 30$
DNA	TBP	26.9	11	29
RNA	Cyclophilin A	28.3	16	35

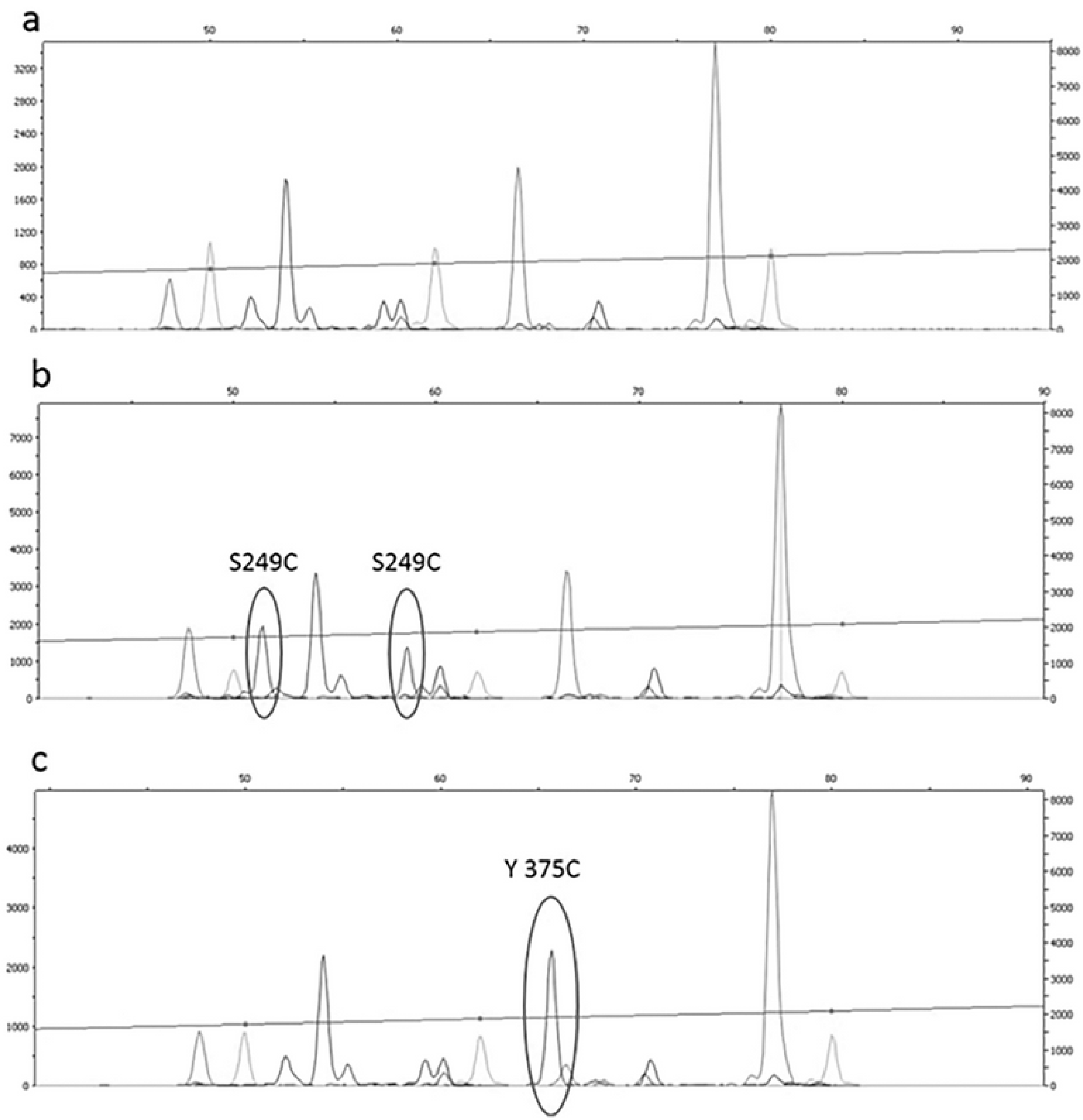

Figure 2
Detection of mutations of FGFR3 in urine DNA samples by SnaPshot assay. (*A*) Wild-type FGFR3. (*B*) S249C mutation. (*C*) Y375C mutation.

The results of the RT-qPCR performed using the reference gene of Cyclophilin A conducted on 15 and 40 RNA extracts provided by healthy volunteers and COBLAnCE patients showed mean Ct of 26.1 (range: 23.4 to 29.2) and 28.5 (range: 22.8 to 35.2), respectively (Table 5). We thus obtained a good amplification of the gene of Cyclophilin A, suggesting that the RNA extracted from urine samples is therefore suitable for its use.

Moreover, Ct of DNA and RNA samples extracted in different time points after urine collection in one healthy volunteer showed that the quality of DNA and RNA was not affected by the period of storage in Norgen tubes at room temperature before extraction (Figure 3).

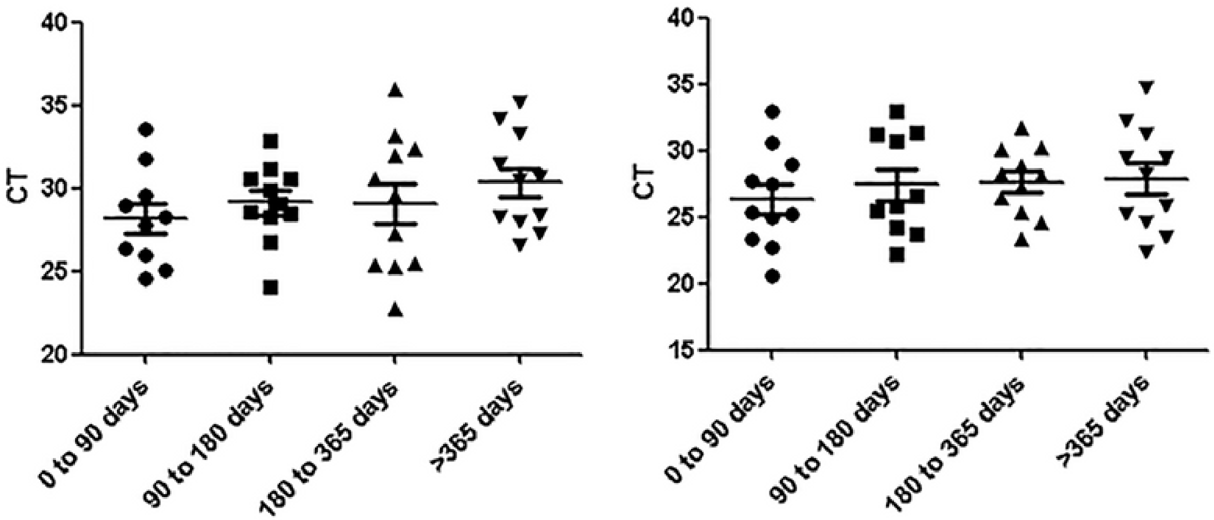

Figure 3
Cycle threshold of RNA (left) and DNA (right) samples extracted in different time points after urine collection.

Concentration and quality of proteins extracted from urine samples

Table 6 shows the protein concentration in the urine extracts. Western blot analysis showed that proteins extracted from urine were not degraded. Specific bands at the molecular weight of Uromodulin (80 kDa), the most abundant protein excreted in ordinary urine, and TSG101 (~45 kDa), an exosomal protein, were detected (Figure 4). The extraction method therefore allowed the precipitation of specifically urinary proteins as Uromodulin, both in the pellet and in the supernatant. We also precipitated vesicle-specific proteins such as TSG101.

Table 6

Concentration of protein samples determined by BCA method

	n	Sample	Median concentration (µg/mL)	Mean yield (µg)
Healthy volunteers	6	Pellet	10,806 (8956-12,500)	5403 (4478-6250)
		Supernatant	4479 (2945-5770)	179 (118-231)

COBLAnCE patients	15	Pellet	8057 (327-15,920)	4029 (163-7960)
		Supernatant	1409 (455-3090)	56 (18-124)

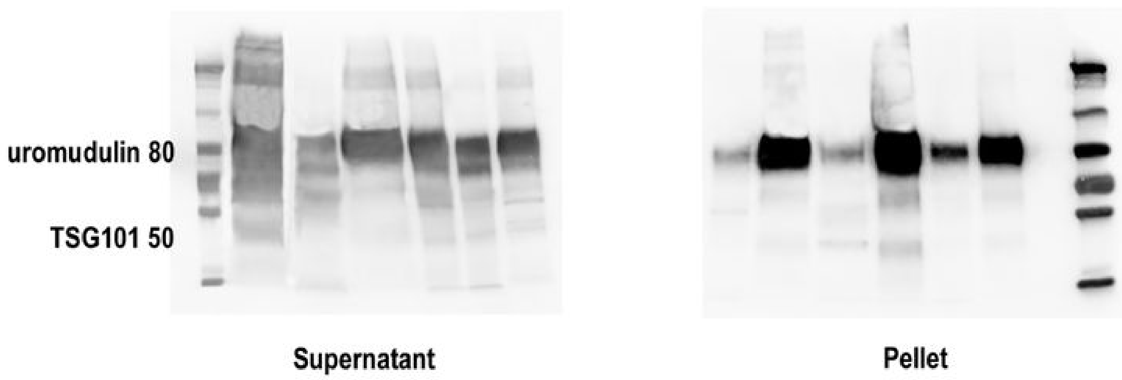

Figure 4
Example of Western blot results obtained in 6 healthy volunteers (on the left) and 8 patients (on the right) attesting the quality of proteins extracted from urine samples.

DISCUSSION

In the present study, we developed efficient extraction techniques on urine to obtain exploitable samples of nucleic acids and proteins for bladder cancer research. Previous publications reported procedures for DNA extraction from urine.[6],[8],[17] Most of them used phenol-chloroform, which is toxic and had a concentration step via sedimentation or diafiltration that is manually laborious and time consuming and therefore not compatible with a large multicentric clinical study. The use of commercial kits has also been described.[1],[9] Regarding the DNA quantity, we obtained a median DNA yield of 4230 ng (110 to 7730 ng) for the female urine versus 560 ng with phenol-chloroform extraction[18] or 12 to 439 ng with commercial kits.[1],[8] A previous report has described a simple procedure for extracting DNA from urine using the Promega Maxwell 16 instrument.[18] The authors obtained low DNA yield (median yield in female samples: 90 ng), probably because of the use of a very small volume of urine (1.7 mL as part of a forensic study). Regarding RNA extraction, there are currently no standard procedures and only few commercially available kits. Our results are comparable to those reported in previous studies (median yield of approximately 800 versus 700 ng, respectively).[4],[19] To our knowledge, there are no reports with automated RNA extraction from urine samples. It should be noted that the nucleic acid extraction techniques developed in the COBLAnCE require less than 1 hour for its completion. It allows relatively high throughput, as 16 samples could be processed simultaneously. Moreover, the semiautomated approach decreases human intervention and minimizes the risks of contamination. Finally, we used the method based on a precipitation with trichloracetic acid described by Court et al.[2]

The present study shows that DNA and RNA recovery is highly variable between patients, with a significant impact of the patient's gender. Because epithelial cells from the genito-urinary tract and leukocytes comprise the primary sources of urinary DNA and RNA,[20] the quantity of nucleic acids varied considerably depending on the sex. Larger amounts of DNA and RNA were recovered from female than male urine samples. This is consistent with the fact that female urine contains more cells and higher amounts of nucleic acids than male urine.[6],[17],[21] Furthermore, variations in the method of urine collection, time of day, and number of urinations may also alter the quantity of nucleic acids in the collected samples.[21] It is well known that less DNA and RNA is available from urine as compared to blood. DNA concentrations obtained in this study are in the same range as that reported by El Bali et al, ranging from 6 to 7128 ng/mL when measured with a spectrophotometric method and from 2 to 274 ng/mL when measured by fluorimetry.[1] The lower concentration measured with fluorimetry compared with spectrophotometry suggests that DNA and RNA obtained are probably highly degraded. Indeed, DNA in urine has been reported to deteriorate quickly.[3] RNA concentrations in COBLAnCE patients were even so weak that they were below the limit of quantification of the assay. Moreover, as pure DNA and RNA have an A260/A280 ratio above 1.7, the low A260/A280 ratios observed suggests that contaminants (eg, substances like sodium azide present in the washing buffer) were not fully removed during extraction. However, this could also result from a very low concentration of nucleic acid. RNA degradation was highlighted by the results obtained by the Agilent Bioanalyzer system analysis. Indeed, RINs were all around 2 to 3, which means that the samples are highly degraded. These results are consistent with those recently reported with a median RIN urine RNA of 2.5 (range: 1.6 to 5.9).[19]

Gene expression analysis based on urine samples is particularly challenging because the nucleic acids are usually more degraded than nucleic acids extracted from other sources. Despite recovery of low amount and fragmented nucleic acids, our PCR experiments performed on the extracts worked well. It was thus possible to use these urine DNA and RNA samples for amplification by PCR, gene expression, or genotyping.

Proper protein extraction and sample preparation are critical to allow suitable Western blotting. Beretov et al studied different protocols, including ultrafiltration, ethanol precipitation, various concentrations of acetone, acetone and trichloracetic acid combination, and combination of trichloric acid and ultracentrifugation.[12] They concluded that methods using trichloracetic acid retain most urinary proteins and allow the best separation and resolution during Western blot. Accordingly, we used this method for urine protein analysis in the COBLAnCE study. Our results showed that uromodulin, a specifically urinary protein, was more abundant in urine supernatant than in the cell pellet, demonstrating the effectiveness of the extraction method used. The detection of TSG101, an exosomal marker, indicates that our extraction protocol allows precipitating exosomal proteins as well. Urinary proteins are promising candidates for bladder cancer diagnosis.[22],[23] Some proteins of urine supernatant could be helpful noninvasive markers for the diagnosis of bladder cancer.[24]

In summary, our results highlighted that a fully automated approach is possible and efficient for urinary DNA and RNA extraction. In addition, we demonstrated that DNA and RNA samples obtained could be used for PCR analysis. Our method using trichloracetic acid is suitable for the purification of urinary proteins and allows urine protein analysis. Urine samples could therefore constitute valuable resources for subsequent investigations in bladder cancer research. Indeed, the discovery of novel proteins and genes and the validity of biomarkers greatly rely on the quality of the nucleic acids and proteins extracted from urine samples.

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